

# CLAIMS

What is claimed is:

- 5           1.       A method for introducing a functional peptide encoded by a plant or protist nucleic acid sequence into a mitochondrion of a mammalian cell, comprising the steps of:
  - (a)     preparing a nucleic-acid construct comprising a plant or protist nucleic acid sequence encoding the peptide and, optionally, a plant or protist nucleic acid sequence encoding a mitochondrial-targeting signal;
  - 10       (b)     introducing the nucleic-acid construct into a mammalian cell to produce a transformed cell; and
  - (c)     expressing the nucleic-acid construct from the nucleus of the transformed cell.
2.       The method of Claim 1, wherein the peptide is a nuclear-DNA-encoded  
15   peptide.
3.       The method of Claim 1, wherein the plant or protist nucleic acid sequence encoding the peptide is an algal nucleic acid sequence.
- 20       4.       The method of Claim 3, wherein the peptide is *Chlamydomonas reinhardtii* ATPase 6 subunit of F<sub>0</sub>F<sub>1</sub>-ATP synthase.
5.       The method of Claim 1, wherein the mitochondrial-targeting signal (MTS) is the MTS of *Chlamydomonas reinhardtii* ATPase 6 subunit of F<sub>0</sub>F<sub>1</sub>-ATP synthase.  
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6.       The method of Claim 1, wherein the mammalian cell is a human cell.
7.       The method of Claim 6, where the cell is a human 293T HEK cell.
- 30       8.       The method of Claim 1, wherein the nucleic-acid construct is introduced into the mammalian cell by a method selected from the group consisting of electroporation, DEAE Dextran transfection, calcium phosphate transfection, cationic liposome fusion,

protoplast fusion, creation of an *in vivo* electrical field, DNA-coated microprojectile bombardment, injection with a recombinant replication-defective virus, homologous recombination, *ex vivo* gene therapy, a viral vector, and naked DNA transfer.

5           9.       The method of Claim 1, wherein the nucleic-acid construct further comprises a nucleic acid sequence encoding a detectable marker.

          10.       The method of Claim 9, wherein the detectable marker is a FLAG epitope.

10          11.       The method of Claim 1, wherein the peptide is *Chlamydomonas reinhardtii* ATPase 6 subunit of F<sub>0</sub>F<sub>1</sub>-ATP synthase and the mammalian cell is a human cell.

          12.       The method of Claim 1, wherein the mammalian cell is in, or is introduced into, a human.

15          13.       The method of Claim 12, wherein the human has a mitochondrial disorder.

          14.       The method of Claim 13, wherein the mitochondrial disorder is associated with a mutation in mtDNA.

20          15.       The method of Claim 14, wherein the mutation is a point mutation.

          16.       The method of Claim 14, wherein the mitochondrial disorder is selected from the group consisting of FBSN (familial bilateral striatal necrosis), NARP (neuropathy, ataxia, and retinitis pigmentosa), and MILS (maternally-inherited Leigh syndrome).

25          17.       The method of Claim 16, wherein the peptide is ATPase 6 subunit of F<sub>0</sub>F<sub>1</sub>-ATP synthase.

30          18.       A method for correcting a phenotypic deficiency in a mammal that results from a mutation in a mitochondrial peptide, comprising the steps of:

          (a)       establishing the identity of the mitochondrial peptide having the mutation;

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(b) preparing a nucleic-acid construct comprising a plant or protist nucleic acid sequence encoding the peptide and, optionally, a plant or protist nucleic acid sequence encoding a mitochondrial-targeting signal, wherein the plant or protist nucleic acid sequence encoding the peptide encodes a functional peptide;

5 (c) introducing the nucleic-acid construct into a mammalian cell to produce a transformed cell; and

(d) expressing the nucleic-acid construct from the nucleus of the transformed cell.

10 19. The method of Claim 18, wherein the peptide is a nuclear-DNA-encoded peptide.

20. The method of Claim 18, wherein the plant or protist nucleic acid sequence encoding the peptide is an algal nucleic acid sequence.

15 21. The method of Claim 20, wherein the peptide is *Chlamydomonas reinhardtii* ATPase 6 subunit of F<sub>0</sub>F<sub>1</sub>-ATP synthase.

22. The method of Claim 18, wherein the mitochondrial-targeting signal (MTS) is the MTS of *Chlamydomonas reinhardtii* ATPase 6 subunit of F<sub>0</sub>F<sub>1</sub>-ATP synthase.

20 23. The method of Claim 18, wherein the mammalian cell is a human cell.

24. The method of Claim 18, wherein the nucleic-acid construct is introduced into the mammalian cell by a method selected from the group consisting of electroporation, DEAE Dextran transfection, calcium phosphate transfection, cationic liposome fusion, protoplast fusion, creation of an *in vivo* electrical field, DNA-coated microprojectile bombardment, injection with a recombinant replication-defective virus, homologous recombination, *ex vivo* gene therapy, a viral vector, and naked DNA transfer.

30 25. The method of Claim 18, wherein the peptide is *Chlamydomonas reinhardtii* ATPase 6 subunit of F<sub>0</sub>F<sub>1</sub>-ATP synthase and the mammalian cell is a human cell.

26. The method of Claim 18, wherein the mammalian cell is in, or is introduced into, a human.

27. The method of Claim 26, wherein the human has a mitochondrial disorder.

28. The method of Claim 27, wherein the mitochondrial disorder is associated with a mutation in mtDNA.

29. The method of Claim 28, wherein the mutation is a point mutation.

30. The method of Claim 28, wherein the mitochondrial disorder is selected from the group consisting of FBSN (familial bilateral striatal necrosis), NARP (neuropathy, ataxia, and retinitis pigmentosa), and MILS (maternally-inherited Leigh syndrome).

31. The method of Claim 30, wherein the peptide is ATPase 6 subunit of  $F_0F_1$ -ATP synthase.

32. A method for treating a mitochondrial disorder in a subject in need of treatment therefore, comprising administering to the subject a functional plant or protist peptide in an amount effective to treat the mitochondrial disorder.

33. The method of Claim 32, wherein the subject is a mammal.

34. The method of Claim 33, wherein the mammal is a human.

35. The method of Claim 32, wherein the peptide is a nuclear-DNA-encoded peptide.

36. The method of Claim 32, wherein the plant or protist is an alga.

37. The method of Claim 36, wherein the peptide is *Chlamydomonas reinhardtii* ATPase 6 subunit of  $F_0F_1$ -ATP synthase.

38. The method of Claim 32, wherein the mitochondrial disorder is associated with a mutation in mtDNA.

5 39. The method of Claim 38, wherein the mutation is a point mutation.

40. The method of Claim 38, wherein the mitochondrial disorder is selected from the group consisting of FBSN (familial bilateral striatal necrosis), NARP (neuropathy, ataxia, and retinitis pigmentosa), and MILS (maternally-inherited Leigh syndrome).

10 41. The method of Claim 40, wherein the peptide is *Chlamydomonas reinhardtii* ATPase 6 subunit of F<sub>0</sub>F<sub>1</sub>-ATP synthase.

15 42. The method of Claim 32, wherein the peptide is administered to the subject by introducing into one or more cells of the subject a nucleic acid sequence encoding the peptide, in a manner permitting expression of the peptide.

43. The method of Claim 32, wherein the peptide is administered to the subject by a method comprising the steps of:

20 (a) obtaining a nucleic acid sequence encoding the peptide;  
(b) preparing a nucleic-acid construct comprising a plant or protist nucleic acid sequence encoding the peptide and, optionally, a nucleic acid sequence encoding a mitochondrial-targeting signal;

25 (c) introducing the nucleic-acid construct into one or more cells of the subject;  
and

(d) in at least one cell of the subject into which the nucleic-acid construct is introduced, expressing the nucleic-acid construct from the nucleus of the cell.

44. The method of Claim 43, wherein step (c) is performed *ex vivo*.

30 45. The method of Claim 43, wherein the peptide is *Chlamydomonas reinhardtii* ATPase 6 subunit of F<sub>0</sub>F<sub>1</sub>-ATP synthase.

46. The method of Claim 43, wherein the mitochondrial-targeting signal (MTS) is the MTS of *Chlamydomonas reinhardtii* ATPase 6 subunit of F<sub>0</sub>F<sub>1</sub>-ATP synthase.

5 47. The method of Claim 43, wherein the nucleic-acid construct is introduced into one or more cells of the subject by a method selected from the group consisting of electroporation, DEAE Dextran transfection, calcium phosphate transfection, cationic liposome fusion, protoplast fusion, creation of an *in vivo* electrical field, DNA-coated microprojectile bombardment, injection with a recombinant replication-defective virus,  
10 homologous recombination, *ex vivo* gene therapy, a viral vector, and naked DNA transfer.

48. An expression vector for use in introducing a functional peptide encoded by an algal nucleic acid sequence into a mitochondrion of a mammal, comprising a nucleic acid sequence encoding *Chlamydomonas reinhardtii* ATPase 6 subunit of F<sub>0</sub>F<sub>1</sub>-ATP synthase or  
15 the mitochondrial-targeting signal thereof.

49. The expression vector of Claim 48, further comprising a nucleic acid sequence encoding a detectable marker.

20 50. The expression vector of Claim 49, wherein the detectable marker is a FLAG epitope.

51. The expression vector of Claim 48, wherein the vector is selected from the group consisting of a bicistronic vector, a plasmid vector, and an adeno-associated virus  
25 (AAV) vector.

52. A mammalian cell transformed by the expression vector of Claim 48.

53. A mammalian cell transformed by the expression vector of Claim 50.

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54. A mammalian cell transformed by an expression vector for use in introducing a functional peptide encoded by a plant or protist nucleic acid sequence into a mitochondrion,

wherein the expression vector comprises a plant or protist nucleic acid sequence encoding the peptide and, optionally, a plant or protist nucleic acid sequence encoding a mitochondrial-targeting signal.

5           55.     The mammalian cell of Claim 54, wherein the cell expresses the peptide.

          56.     The mammalian cell of Claim 54, which is a human cell.

          57.     The mammalian cell of Claim 54, which is selected from the group consisting  
10 of a clonal cell, a stem cell, and a progenitor cell.

          58.     The mammalian cell of Claim 54, wherein the peptide is a nuclear-DNA-encoded peptide.

15           59.     The mammalian cell of Claim 54, wherein the plant or protist nucleic acid sequence encoding the peptide is an algal nucleic acid sequence.

          60.     The mammalian cell of Claim 59, wherein the peptide is *Chlamydomonas reinhardtii* ATPase 6 subunit of F<sub>0</sub>F<sub>1</sub>-ATP synthase.

20           61.     The mammalian cell of Claim 54, wherein the mitochondrial-targeting signal (MTS) is the MTS of *Chlamydomonas reinhardtii* ATPase 6 subunit of F<sub>0</sub>F<sub>1</sub>-ATP synthase.

          62.     The mammalian cell of Claim 54, wherein the expression vector transforms  
25 the cell by a method selected from the group consisting of electroporation, DEAE Dextran transfection, calcium phosphate transfection, cationic liposome fusion, protoplast fusion, creation of an *in vivo* electrical field, DNA-coated microprojectile bombardment, injection with a recombinant replication-defective virus, homologous recombination, *ex vivo* gene therapy, a viral vector, and naked DNA transfer.

30           63.     The mammalian cell of Claim 54, wherein the expression vector further comprises a nucleic acid sequence encoding a detectable marker.

64. The mammalian cell of Claim 63, wherein the detectable marker is a FLAG epitope.

65. The mammalian cell of Claim 54, wherein the expression vector is selected from the group consisting of a bicistronic vector, a plasmid vector, and an adeno-associated virus (AAV) vector.

66. A clonal cell strain comprising the transformed mammalian cell of Claim 54.

67. A pharmaceutical composition, comprising:

- (a) a plant or protist nucleic acid sequence encoding a peptide for introduction into a mitochondrion;
- (b) optionally, a plant or protist nucleic acid sequence encoding a mitochondrial-targeting signal; and
- (c) a pharmaceutically-acceptable carrier.

68. The pharmaceutical composition of Claim 67, wherein the peptide is a nuclear-DNA-encoded peptide.

69. The pharmaceutical composition of Claim 68, wherein the plant or protist nucleic acid sequence encoding a peptide for introduction into a mitochondrion is an algal nucleic acid sequence.

70. The pharmaceutical composition of Claim 69, wherein the peptide is *Chlamydomonas reinhardtii* ATPase 6 subunit of F<sub>0</sub>F<sub>1</sub>-ATP synthase.

71. The pharmaceutical composition of Claim 67, wherein the mitochondrial-targeting signal (MTS) is the MTS of *Chlamydomonas reinhardtii* ATPase 6 subunit of F<sub>0</sub>F<sub>1</sub>-ATP synthase.



72. A method for introducing a functional peptide into a mitochondrion, comprising the steps of:

- (a) preparing a nucleic-acid construct comprising a nucleic acid sequence encoding the peptide and a nucleic acid sequence encoding the mitochondrial-targeting sequence of *Chlamydomonas reinhardtii* ATPase 6 subunit of F<sub>0</sub>F<sub>1</sub>-ATP synthase;
- (b) introducing the nucleic-acid construct into a eukaryotic cell to produce a transformed cell, wherein the eukaryotic cell is derived from an animal, a plant, a fungus, or a protozoan; and
- (c) expressing the nucleic-acid construct from the nucleus of the transformed cell.

73. The method of Claim 72, wherein the peptide is encoded by mitochondrial DNA.

74. The method of Claim 73, further comprising the step of modifying the mitochondrial DNA (mtDNA), if necessary, before step (a), to render the mtDNA compatible with the universal genetic code.

75. The method of Claim 74, wherein the peptide is selected from the group consisting of apocytochrome b, an ATP synthase F<sub>1</sub> subunit, an ATP synthase F<sub>0</sub> subunit, a cytochrome *c* oxidase subunit, DNA polymerase, elongation factor, a haem lyase subunit, a NADH dehydrogenase subunit, an L ribosomal protein, an S ribosomal protein, RNA polymerase, an RNA polymerase subunit, reverse transcriptase, and succinate dehydrogenase subunit.

76. The method of Claim 75, wherein the peptide is human ATPase 6 subunit of F<sub>0</sub>F<sub>1</sub>-ATP synthase.

77. The method of Claim 72, wherein the peptide is a nuclear-DNA-encoded peptide.

78. The method of Claim 77, wherein the peptide is selected from the group consisting of an ATP synthase F<sub>1</sub> subunit, an ATP synthase F<sub>0</sub> subunit, a cytochrome *c* oxidase subunit, and an L ribosomal protein.

5 79. The method of Claim 72, wherein the nucleic-acid construct is introduced into the eukaryotic cell by a method selected from the group consisting of electroporation, DEAE Dextran transfection, calcium phosphate transfection, cationic liposome fusion, protoplast fusion, creation of an *in vivo* electrical field, DNA-coated microprojectile bombardment, injection with a recombinant replication-defective virus, homologous recombination, *ex vivo* gene therapy, a viral vector, and naked DNA transfer.

80. The method of Claim 72, wherein the nucleic-acid construct further comprises a nucleic acid sequence encoding a detectable marker.

15 81. The method of Claim 80, wherein the detectable marker is a FLAG epitope.

82. The method of Claim 72, wherein the eukaryotic cell is a mammalian cell.

83. The method of Claim 82, wherein the cell is a human cell.

20 84. The method of claim 83, wherein the cell is a human 293T HEK cell.

85. The method of Claim 82, wherein the eukaryotic cell is in, or is introduced into, a mammal.

25 86. The method of Claim 85, wherein the mammal is a human.

87. The method of Claim 86, wherein the human has a mitochondrial disorder.

30 88. The method of Claim 87, wherein the mitochondrial disorder is associated with a mutation in mtDNA.

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89. The method of Claim 88, wherein the mutation is a point mutation.

90. The method of Claim 88, wherein the mitochondrial disorder is selected from  
the group consisting of FBSN (familial bilateral striatal necrosis), NARP (neuropathy, ataxia,  
5 and retinitis pigmentosa), and MILS (maternally-inherited Leigh syndrome)

91. The method of Claim 90, wherein the peptide is human ATPase 6 subunit of  
F<sub>0</sub>F<sub>1</sub>-ATP synthase.